

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 7/20	A1	(11) International Publication Number: WO 97/07777 (43) International Publication Date: 6 March 1997 (06.03.97)
(21) International Application Number: PCT/US96/13240 (22) International Filing Date: 15 August 1996 (15.08.96) (30) Priority Data: 60/002,361 15 August 1995 (15.08.95) US 60/012,537 29 February 1996 (29.02.96) US (71)(72) Applicant and Inventor: MONTGOMERY, Robert, Eric [US/US]; 29 Fairview Road, P.O. Box 487, Monterrey, MA 01245 (US). (74) Agents: SUNSTEIN, Bruce, D. et al.; Bromberg Sunstein, 125 Summer Street, Boston, MA 02110-1618 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PEROXIDASE-ACTIVATING ORAL COMPOSITIONS (57) Abstract An oral care composition and method of activating peroxidase in the oral cavity of an animal is provided. The composition includes a non-enzymatic water-soluble hydrogen peroxide precursor. This precursor is capable of releasing hydrogen peroxide upon contact with water to activate the peroxidase system. The composition further contains a pH-adjusting agent to produce a selected pH that facilitates the rapid release of hydrogen peroxide from the precursor which then is available to activate the peroxidase system in the oral cavity. The method involves the administration of a mixture of a non-enzymatic water-soluble hydrogen peroxide precursor and pH adjusting agent. A process for manufacturing an oral care product is also provided that utilizes an alkali metal percarbonate as hydrogen peroxide precursor.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

PEROXIDASE-ACTIVATING ORAL COMPOSITIONS

Technical Field

This present invention relates to a method for activating a peroxidase
5 enzyme system in situ.

Cross Reference

This application claims priority from provisional applications No
60/002,361 and 60/012,537.

10

Background Art

A number of naturally occurring antimicrobial systems rely upon the
ability of certain oxidizing agents to disrupt metabolic processes of bacteria, fungi
and viruses. Examples of such oxidizing agents include hypothiocyanite (OSCN-
15 / HOSCN), hypochlorite (OCl- \ HOCl), and hypoiodite (OI- \ HOI). These
agents are known to inhibit glycolysis, penetrate prokaryotic cell walls, and
generally disrupt a wide variety of processes crucial to the survival of lower
organisms at concentrations greater than or equal to about 100 micromoles per
liter. The oxidizing agents are formed from the detoxification of hydrogen
20 peroxide by mammalian peroxidase systems, such as those found in saliva,
cervical fluid, lachrymal fluid, and leukocytes. Examples of such peroxidase
system enzymes are myeloperoxidase, lactoperoxidase, and salivary peroxidase.

Attempts to exploit these natural antimicrobial systems have been directed
to both the oral care field and the gastrointestinal tract. US 4,150,113 and US
25 4,178,362 (Hoogendorn, et al.) describe dentifrice compositions containing
glucose oxidase that react with plaque and salivary glucose to produce low levels
of hydrogen peroxide. Hydrogen peroxide production by such systems is,
however, highly irregular due to the non-uniform distribution and unpredictable
availability of substrate, namely glucose, in the oral cavity.

30 US 4,269,822, US 4,564,519 and US 4,578,265 (Pellico, et al.) further describe

dentifrice compositions containing an oxidoreductase enzyme and its specific substrate in an aqueous solution for the purpose of producing hydrogen peroxide or other antimicrobial oxidizing compounds such as hypothiocyanite ion. A more predictable amount of hydrogen peroxide (and subsequently
5 hypothiocyanite ions) is produced by the compositions of Pellico et al., compared with those of the Hoogendorn references. The differences between the two compositions reflect the availability of glucose in the oral cavity as substrate for glucose oxidase.

There are, however, a number of disadvantages associated with the
10 compositions of Pellico et al. These include: the limited rate of enzymatically-produced hydrogen peroxide that in turn produces the hypothiocyanite ion. The short duration of oral contact time, namely during toothbrushing, means that insufficient amounts of hypothiocyanite is available to effectively eliminate microbes in the oral cavity. In addition, the references utilize glucose oxidase as
15 the oxidoreductase enzyme that in turn relies upon the availability of a sufficient concentration of glucose in solution to produce hydrogen peroxide. However, the glucose itself is a microbial substrate and is potentially cariogenic when present in an oral care product.

US 4,564,519 describes a chewable dentifrice, such as a chewing gum or
20 lozenge, which contains a dual enzyme system for producing hypothiocyanite ions upon being chewed or otherwise activated by the moisture in saliva. Such compositions suffer from similar drawbacks to those mentioned immediately above namely a slow rate of enzymatically-produced hydrogen peroxidase as well as a reliance on a cariogenic compound.

25 Other solid or chewable compositions capable of producing hydrogen peroxide or other oxidizing agents upon activation with moisture are taught in US 4,320,116, US 4,726,948, and US 4,929,466. These compositions are foodstuffs intended for consumption by livestock in order to limit the growth of harmful bacterial within the animal's gastrointestinal tract. These references describe the
30 use of various enzymatic and non-enzymatic sources for hydrogen peroxide,

- 3 -

where the enzymatic sources are glucose oxidase / glucose and the non-enzymatic sources are sodium perborate, sodium percarbonate, and calcium peroxide. However, it is known that sodium percarbonate and potassium percarbonate have extremely alkaline pH and are thus of little use in activating
5 the peroxidase enzymes until exposed to the acidic environment within the gastrointestinal tract. Thus, the foodstuff compositions described in the reference cannot be used as a therapeutic or otherwise peroxidase-activating effect in the oral cavity.

It would thus be advantageous to provide substantially non-cariogenic
10 compositions capable of rapidly producing hydrogen peroxide in conditions that are suitable for peroxidase enzyme activation in the oral cavity.

It would also be advantageous to provide compositions capable of rapidly producing antimicrobial hypohalite ions within the limited contact time available in most oral hygiene procedures.

15 It would also be advantageous to provide compositions capable of rapidly producing antimicrobial hypohalite ions upon contact with saliva within the limited contact time available in most oral hygiene procedures.

Summary

20 This invention satisfies the above needs. A novel oral care composition is provided.

A preferred embodiment of the invention is a non-enzymatic, water-soluble hydrogen peroxide precursor, capable of rapidly releasing an effective amount of hydrogen peroxide for activating the peroxidase system in the oral
25 cavity, upon contact with an aqueous solution; and a pH adjusting agent capable of producing a selected pH in the aqueous solution for facilitating the rapid release of the hydrogen peroxide from the hydrogen peroxide precursor and the activation of the peroxidase enzyme in the oral cavity.

In a further embodiment of the invention, a process is provided for
30 manufacturing an oral care product, comprising the steps of obtaining an alkali

metal percarbonate; dispersing the percarbonate in a non-hygroscopic material so as to encapsulate the percarbonate; obtaining particles of percarbonate encapsulated in the non-hygroscopic material; associating the percarbonate particles with a pH adjusting agent; and formulating the particles into an oral
5 care product.

In a further embodiment of the invention, a method is provided for activating a peroxidase system in an oral cavity of an animal, including the steps of selecting a non-enzymatic water soluble hydrogen peroxide precursor capable of rapidly releasing an effective amount of hydrogen peroxide for activating the
10 peroxidase system in the oral cavity upon contact with an aqueous solution; mixing the precursor with a pH adjusting agent capable of producing a selected pH in the aqueous solution for facilitating the rapid release of the hydrogen peroxide from the hydrogen peroxide precursor and the activation of the peroxidase enzyme in the oral cavity; and administering to the oral cavity the
15 precursor and pH-adjusting agent in a suitable formulation.

Detailed Description of the Invention

The present invention relates to oral care compositions which upon contact with an aqueous solution, are capable of rapidly activating a peroxidase
20 enzyme so as to release hydrogen peroxide.

The concentrations of the components of the oral composition are given in molar units which denote the concentration of the component in the aqueous contact solution.

The limiting factor in all of the mammalian antimicrobial peroxidase
25 systems is the availability of the substrate, namely hydrogen peroxide.

Furthermore, the pH of the aqueous environment determines not only the effective release of hydrogen peroxide from the precursor but also the activity of the peroxidase system and the efficacy of the resulting oxidizing agents in penetrating the cell walls of microorganisms.

30 It is known, for example, that the non-ionized species of hypohalite ions

more readily penetrates the cell walls of microorganisms than does the ionized species thereby having increased efficiency in inhibiting the metabolism of the microorganisms. The distribution of ionized versus non-ionized species (for instance HOCl, or hypochlorous acid, versus OCl⁻, or hypochlorite ion) is highly
5 pH dependent.

The pH activity profiles of the peroxidase enzymes lactoperoxidase, salivary peroxidase, and myeloperoxidase is maximum between pH 5 and 6, but drop off sharply below pH 4.0 and above pH 7.5. Thus, in order to maintain peroxidatic function, it is here concluded that the pH of the medium surrounding
10 the peroxidase enzymes must be within the range of about pH 4.0 to about pH 7.5. This pH range also favors the anti-microbial, non-ionized hypohalite species which prevail at lower pH levels.

Consequently, the compositions of the invention include a non-enzymatic water-soluble hydrogen peroxide precursor and a water-soluble pH adjusting
15 component capable of providing a pH to an aqueous contact solution of between about 4.0 and 7.9 The aqueous contact solution may commonly be saliva, but may also include an aqueous solution that is mixed with the precursor and pH adjusting agent prior to contact with the oral cavity.

The non-enzymatic water-soluble precursor may be selected from the
20 group of stable persalts including, but not limited to, alkali metal percarbonates, for example, sodium and potassium percarbonate, alkali metal perborates, alkali metal peroxides, and hydrogen peroxide complexes such as carbamide peroxide. Preferred non-enzymatic hydrogen peroxide precursors are sodium percarbonate and carbamide peroxide due to their solubility characteristics and relatively
25 benign toxicity in limited concentrations. The most preferred non-enzymatic hydrogen peroxide precursor is sodium percarbonate.

Sodium percarbonate is a relatively stable complex containing 2 moles of sodium carbonate complexed with 3 moles of hydrogen peroxide (27% hydrogen peroxide by weight). It is highly water soluble (120 grams per liter at 20° C) and
30 produces a pH upon dissolution of between 10 and 11 (for a 1% solution). Thus,

although sodium percarbonate possesses the desirable hydrogen peroxide-releasing properties for the practice of the present invention, alone they are of little utility for the activation of a peroxidase enzyme due to their high in-solution pH properties. Accordingly, a pH adjusting agent has been utilized to

5 normalize the pH to a range of 4.0-7.9.

Carbamide peroxide is a 1 to 1 molar complex between urea and hydrogen peroxide (35% hydrogen peroxide by weight) with a molecular weight of 94.07. It is usually manufactured in the form of crystals which are highly soluble in water (800 grams per liter of water at 20° C to yield a saturated solution of 44.4%

10 carbamide peroxide, equivalent to a hydrogen peroxide concentration of 15.5%). However, when carbamide peroxide is solubilized in water, a pH of approximately 3.40 (for a saturated solution) to approximately 4.05 (for a 1% solution) is obtained. This pH is slightly below the desirable range, according to the invention, for activating a peroxidase enzyme in the aqueous contact solution

15 absent a pH adjusting agent.

The pH adjusting agent of the present invention may include any toxicologically acceptable and preferably water-soluble ingredient which is capable of producing an aqueous contact solution pH of between about 4.0 and about 7.5. Most preferably, the pH adjusting agent will provide a pH of between

20 5.0 and 6.0. Such pH adjusting agents include a wide variety of common buffers, acidulants, and/or alkalizers which are well known to those skilled in the art. Examples include organic acids and their alkali metal salts, such as citric acid, malic acid, butyric acid, gluconic acid, adipic acid, glutaric acid, and malonic acid; amines such as triethanolamine and tris(hydroxyaminomethane); alkali

25 metal hydroxides, such as sodium hydroxide, potassium hydroxide, and ammonium hydroxide; and combinations thereof. Preferably, these pH adjusting agents are free of water of hydration in order to achieve long-term stability in the presence of the hydrogen peroxide precursor. The optimum concentration of the pH-adjusting agent is the lowest level necessary to achieve the desired pH

30 adjustment of the aqueous contact solution to between 4.0 and 7.5. This

concentration of the pH adjusting agent is in the range of about 0.01% by weight of the composition to about 1% by weight of the composition. However, higher and lower amounts may have utility in circumstances where the buffering capacity of the surrounding medium is either very strong or very weak,
5 respectively.

The oral care composition described above generates hydrogen peroxide to be used as substrate for the peroxidase system in a manner that permits the concentration of hydrogen peroxide generated overall to be reduced while increasing the rate of its production. This provides a safe and effective
10 antimicrobial composition. While the prior art describes antimicrobial activity associated with oral care products containing or generating hydrogen peroxide, the present invention provides for the first time the particular advantages of both limiting the concentration of hydrogen peroxide to a level which is suitable for the activation of a peroxidase enzyme (less than about 10 millimoles per liter *in*
15 *situ*) and providing or limiting the pH range of the *in situ* composition/saliva fluids to that which is also most advantageous for activation of a peroxidase enzyme.

The antimicrobial activity of the oral compositions depend on the presence of an oxygen acceptor. The preferred oxygen acceptor for oral care applications
20 (i.e., in the presence of salivary peroxidase or lactoperoxidase) is the thiocyanate ion, which can be provided to the composition through the inclusion of non-toxic levels of a thiocyanate salt, such as potassium or sodium thiocyanate. In general, the level of thiocyanate salt included in said compositions will be from about 1.0 millimolar to about 10.0 millimolar (again, as above, based upon the
25 concentration achieved in the aqueous contact solution). However, the composition of the invention may be further enhanced by incorporating halide ions in the aqueous contact solution. One or more oxygen-accepting halide or pseudohalide ions, including any of chloride, iodide, bromide, and thiocyanate and combinations thereof, may be incorporated in the aqueous contact solution.
30 These ions may already be present in solution (such as thiocyanate ion, which is

present in saliva), or alternatively they may be provided as auxiliary components in the inventive compositions.

In general, oral care product use results in a dilution of composition components on the order of 1 part composition to from about 1 part aqueous contact solution to about 5 parts aqueous contact solution (from about 1 to 1 to about 1 to 5). It is desirable that the non-enzymatic hydrogen peroxide precursors should be present at a level sufficient to release a minimum amount of hydrogen peroxide of approximately 100 micromoles per liter. A preferred range of hydrogen peroxide released is in the range of from about 500 micromoles per liter to about 2,000 micromoles per liter. The determination of the amounts of hydrogen peroxide that are released from a given composition *in vitro* (for instance, under controlled conditions in contact with distilled water as a diluent) are relatively simple to predict. However, the determination of amounts of hydrogen peroxide released *in vivo* indicates that *in vivo* levels are well below those predicted *in vitro*. This difference may result from the destruction of hydrogen peroxide by salivary catalase, interaction of hydrogen peroxide with various organic matter and non-enzymatic reducing agents in saliva, and the destructive effect of dissolved metal ions in saliva. Thus, while an upper limit of 2.0 millimoles of hydrogen peroxide per liter is predictive of an *in vitro* effectiveness, concentrations as high as 30 millimoles per liter may be desirable to produce much lower observed *in vivo* concentrations of hydrogen peroxide.

It should also be noted that an accumulation of high concentrations of hydrogen peroxide (greater than about 0.1 percent, or about 29 millimoles per liter) are not desirable due to the evidence that hydrogen peroxide is mutagenic and can cause cellular DNA damage at elevated concentrations. The prior art describes broad concentration ranges of hydrogen peroxide as having utility in oral hygiene and tooth whitening applications, but makes little reference to the potential harmful effects of hydrogen peroxide at concentrations, for instance, in the range of 1.5 to 3.0 percent by weight (441 to 882 millimolar).

Although the peroxidase enzyme may typically be present in the aqueous

contact solution (such as salivary peroxidase, which is present in saliva), additional peroxidase enzyme, preferably lactoperoxidase, may be included in the inventive compositions in a range of from about 10 ABTS (2,2'-Azinobis(3-ethylbenzthiazoline sulfonic acid) units per gram of composition to about 1,000
5 ABTS units per gram of composition [under the assay conditions described in Pruitt, et al., Analytical Biochemistry 191, pp. 278-286 (1990)].

In a preferred embodiment of the invention, compositions may be non-aqueous, dry, or otherwise substantially water-free mixtures, which can be applied or deposited on or within an orally acceptable carrier, such as a chewing
10 gum, dental floss, anhydrous dentifrice, or animal chew. These compositions, once dissolved in the aqueous solution at a selected pH, are capable of producing, in the absence of additional enzyme preparations, hydrogen peroxide that results in the rapid activation of an antimicrobial peroxidase enzyme system *in vivo*.

15 The hydrogen peroxide precursors of the invention, such as the alkali metal percarbonates, may be processed with little or no loss of activity from moisture pick-up if, prior to being deposited onto or into an orally acceptable carrier, the alkali metal percarbonate is first dispersed in a non-hygroscopic fluid or solid in order to coat or encapsulate each particle of the percarbonate prior to
20 being entered into a manufacturing process. Alkali metal percarbonates processed in this manner show very little degradation during processing cycles due to moisture absorption and/or temperature exposure. Although the concept of coating moisture-sensitive materials with water-insoluble or non-hygroscopic outer layers is not new to the art, the inventive aspect of the present invention
25 stems from the requirement that said coating must necessarily consist of a moisture-resistant fluid which has mobility and will form a fluid interface in contact with bulk moisture. Only through the formation of a thin interfacial layer in contact with bulk moisture will the intimately admixed alkali metal percarbonate and non-hygroscopic fluid release the alkali metal percarbonate (as
30 hydrogen peroxide and an alkali metal carbonate) into the neighboring aqueous

- 10 -

phase.

Preferred non-hygroscopic fluids and solids are non-solvents for alkali metal percarbonates. These fluids are water-insoluble, yet low enough in viscosity to be readily dispersed into a thin film or interface in the presence of

5 bulk moisture. Such non-hygroscopic liquids include, but are not limited to, mineral oils, vegetable oils, fatty esters, silicone fluids, fluorinated hydrocarbons, and fluorosilicones. Preferred non-hygroscopic solids are also water insoluble and must be capable of being melt processed at a temperature suitable for maintaining the stability of the alkali metal percarbonate. Suitable non-

10 hygroscopic solids include, but are not limited to, waxy solids such as mineral oils, vegetable oils, fatty esters, silicone fluids, fluorinated hydrocarbons, fluorosilicones, stearic acid, glyceryl monostearate, paraffin wax, microcrystalline wax, and fatty alcohols. An example of an applicable melt process includes batch melt processing, whereby the non-hygroscopic solid carrier is simply melted in

15 bulk, whereupon the alkali metal percarbonate is added and dispersed. Another example of an applicable melt process is the fluid-bed processing technique described in US Patent No. 4,421,669, whereby the alkali metal percarbonate (in the form of a powdered or granular particle) is floated on an airstream and subsequently sprayed with melted droplets of the non-hygroscopic solid carrier.

20 Both non-hygroscopic fluid and solid carriers may also include the pH-adjusting agents of the present invention, so as to simplify the application of both inventive components (i.e., the hydrogen peroxide precursor and the pH-adjusting agent) onto or into the oral care product delivery system.

Alternatively, the hydrogen peroxide precursor and the pH-adjusting agent may

25 be dispersed separately within two different non-hygroscopic carriers and subsequently applied onto or into the oral care product delivery system in a stepwise fashion.

The choice of non-hygroscopic fluid or solid carrier for the alkali metal percarbonate is dependent upon the final oral care product delivery system

30 contemplated. Where the formulation is an animal chew, such as a rawhide

EXAMPLES

15 The components of 8 compositions were added to a laboratory powder
blender and mixed for 1 hour in order to assure complete mixing. The resulting
white powders were extremely fine and free flowing.

Each sample 1A - 1H (100mg) was contacted with 100 ml distilled water at 25°C and the resulting hydrogen peroxide concentration after 15 seconds [recorded as H₂O₂ (t=15 sec)] was determined by the method of Mottola, et al., Analytical Chemistry 42, pp. 410 - 411 (1970). The pH at 25° C of each solution [recorded as pH (t=15 sec)] at the 15 second point was also determined. The results are recorded in Table 2 below.

25 Table 1: A comparison of 8 compositions (wt in gms)

[illegible]

Fresh, whole unstimulated human saliva was collected from 3 subjects drooling into chilled plastic cups, said cups being placed in ice during and after the expectoration procedure, and the 3 samples of saliva pooled. A sample of the freshly-pooled saliva was warmed to 37°C and evaluated for its hypothiocyanite ion concentration [recorded as OSCN (initial)] by the method of Mansson-Rahemtulla, et al., Archives of Oral Biology 31, 10, pp. 661-668 (1986). Readings recorded as "OSCN" are intended to reflect the combined concentration of OSCN- ion and the non-ionized HOSCN species. The powdered compositions of the present example were then contacted with the pooled saliva at a concentration of 1.0 mg of powder in 1.0 ml of saliva. After 15 seconds, the saliva was reevaluated for its hypothiocyanite ion concentration as above [recorded as OSCN (t=15 sec)] and the results recorded in Table 2 below.

15

Table 2: hypothiocyanite concentrations in saliva

SAMPLE	1A	1B	1C	1D	1E	1F	1G
H ₂ O ₂ (t=15 sec) in micromoles/liter	750	765	760	755	760	760	765
pH (t=15 sec) @ 37 degrees C	9.17	7.95	7.20	6.10	4.89	3.82	3.10
OSCN (initial) in micromoles/liter	42	42	42	42	42	42	42
OSCN (t=15 sec) in micromoles/liter	19	114	160	269	257	199	58

25

It can be seen from the above that the powdered compositions of Table 1 which result in a solution pH of from about 3.82 to 7.95 are capable of rapidly elevating the concentration of hypothiocyanite ions in pooled, unstimulated whole human saliva. Supplementation of the mannitol in Table 1 with potassium thiocyanate (at a level capable of providing a 1.0 millimolar concentration of thiocyanate ion in the pooled saliva sample) resulted in an even more dramatic increase in the observed hypothiocyanite ion concentration, probably due to the reaction-limiting concentration of naturally occurring thiocyanate in the pooled saliva sample.

30

EXAMPLE II: A comparison of hydrogen peroxide concentrations produced *in vivo* and *in vitro*.

The composition of Sample 1D was combined, in different proportions, with pooled saliva in order to produce pH-adjusted saliva containing varying concentrations of hydrogen peroxide [recorded as H₂O₂ (actual)]. The theoretical concentration of hydrogen peroxide is also shown [recorded as H₂O₂ (theoretical)]. The concentrations of OSCN produced are also recorded in Table 3 below. All measurements were made at t = 15 seconds after mixing the prescribed weight of Sample 1D with saliva.

Table 3 :

EXAMPLE 1D (MG / LITER)	H ₂ O ₂ (theoretical) (MICROMOLES/ LITER)	H ₂ O ₂ (actual) (MICROMOLES/ LITER)	pH	OSCN (MICROMOLES/ LITER) NO ADDED SCN-	OSCN (MICROMOLES /LITER) SCN- @ 1 MILLIMOLAR
10	7.94	< 1	5.86	38	42
100	79.4	< 1	6.02	88	87
1,000	794	52	6.10	285	420
2,000	1,588	120	6.18	321	598
4,000	3,176	2,185	6.20	339	712
6,000	4,764	3,890	6.25	301	690
10,000	7,941	6,920	6.25	233	662
20,000	15,882	13,202	6.26	110	272
40,000	31,764	29,990	6.25	93	160
100,000	79,410	78,540	6.25	37	90

EXAMPLE III: Hypothiocyanite ion concentrations resulting from an oral composition on chewing gum

The composition of sample 1D was applied to the surface of 3.0 gram sticks of chewing gum at a coating rate of 0.05% by weight of gum in order to produce chewing gum which was capable of generating hydrogen peroxide upon contact with saliva, in addition to providing for a salivary pH adjustment to about 6.0.

One stick of Sample 1D-coated chewing gum (3.05 grams) was broken into small pieces and vortexed with 3.05 grams of distilled water for 15 seconds. At exactly the 15 second point, a sample of the resulting fluid was assayed for

hydrogen peroxide and pH as in Example I. The fluid contained a hydrogen peroxide concentration of 360 micromolar and the fluid pH was 6.03.

Saliva samples from five subjects, ages 25 - 45, were collected as above, but rather than being pooled, were assayed individually for hypothiocyanite ion concentrations. The results are recorded below. The same volunteers were then asked to chew the coated chewing gum samples for a period of 2 minutes. Their saliva was collected again, and their salivary hypothiocyanite ion levels recorded. (Table 4). The results show a remarkable ability of the inventive composition to impart salivary peroxidase-activating properties to the chewing gum.

10

Table 4

	SUBJECT	OSCN BEFORE	OSCN AFTER
	1	29 micromolar	214 micromolar
15	2	56 micromolar	198 micromolar
	3	45 micromolar	260 micromolar
	4	39 micromolar	252 micromolar
	5	28 micromolar	208 micromolar

20

EXAMPLE IV : **Hydrogen peroxide concentrations resulting from an oral composition on a rawhide chew**

A rawhide animal chew was prepared by taking 10 pounds of dried, unbasted rawhide chews, approximately 2 inches wide by 6 inches in length, and spray coating them at a 1.0 percent coating rate with the following composition;

Table 5

	COMPONENT	AMOUNT
30	Light Mineral Oil USP	87 grams
	Sodium Percarbonate (Solvay - FB100)	10 grams
	Malic Acid (Powder FCC)	3 grams
	TOTAL	100 grams

35

The above components were slurried until a fine dispersion of solids was

- 15 -

obtained. Agitation continued during the spray process to prevent the settling out of the solids. The sprayed rawhide chews were dried at room temperature for 24 hours, during which time the initial surface gloss observed on the freshly sprayed chews disappeared.

- 5 In order to determine the ability of the spray-coated rawhide chew to generate pH- adjusted hydrogen peroxide upon contact with water, single chews cut into four pieces and weighed. An equivalent amount of distilled water was weighed out and the coated chews vortexed in the water for 15 seconds. The "chew fluid" contained a hydrogen peroxide concentration of 6.53 millimolar at a
10 pH of 5.84 at 25°C.

EXAMPLE V: Delivery of the oral composition in a gel

- An anhydrous carbamide peroxide gel composition was prepared in order
15 to demonstrate another option for delivery of the composition to the oral cavity.

Table 6

	COMPONENT	AMOUNT
20	Glycerine 99.7% USP	93.45 grams
	Carbopol 980 NF (BF Goodrich)	2.00 grams
	Carbamide Peroxide (Degussa) USP	0.05 grams
	Distilled Water	3.00 grams
	Tris(hydroxymethyl)aminomethane USP	1.50 grams
25	TOTAL	100 grams

- The Carbopol 980 NF was dispersed under high shear in the Glycerine 99.7% USP and subsequently deaerated. The Carbamide Peroxide was then dissolved in this mixture under low shear mixing. The
30 Tris(hydroxymethyl)aminomethane was dissolved in the Distilled Water, and this phase dispersed into the main phase under 28" Hg vacuum in order to avoid entrapment of air. The resulting gel was highly viscous and transparent.

 In the above composition, the tris (hydroxymethyl) aminomethane USP

- 16 -

serves as both a neutralizer for thickening the acidic carboxypolymethylene (Carbopol 980 NF) and as an alkalizer to provide a suitable peroxidase-active pH during the use of this product. The pH of a 1:5 dilution (1 part Example V to 5 parts Distilled Water) is 5.4, and the dilution showed a hydrogen peroxide
5 concentration of 969 micromoles per liter.

I claim:

1. An oral care composition for activating a peroxidase system in an animal oral cavity, comprising:
 - a non-enzymatic, water-soluble hydrogen peroxide precursor
 - 5 capable of rapidly releasing an effective amount of hydrogen peroxide for activating the peroxidase system in the oral cavity upon contact with an aqueous solution; and
 - a pH-adjusting agent capable of producing a selected pH in the aqueous solution for facilitating the rapid release of the hydrogen peroxide from
 - 10 the hydrogen peroxide precursor, and the activation of the peroxidase enzyme in the oral cavity.
2. The composition according to claim 1, wherein the peroxide precursor is selected from the group consisting of: alkali metal percarbonates;
- 15 alkali metal perborates, alkali metal peroxides, and hydrogen peroxide precursor complexes.
3. The composition of claim 2, wherein the alkali metal percarbonate is sodium percarbonate.
- 20 4. The composition of claim 2, wherein the hydrogen peroxide precursor complex is carbamide peroxide.
5. The composition according to claim 1, wherein the effective amount
- 25 of hydrogen peroxide is greater than 100 micromoles per liter.
6. The composition according to claim 5, wherein the effective amount of hydrogen peroxide is in the range of 100 micromoles per liter to 30 millimoles per liter.

7. The composition according to claim 6, wherein the effective amount of hydrogen peroxide is in the range of 100 micromoles per liter to about 2000 micromoles per liter.

5 8. The composition according to claim 1, wherein the effective amount of hydrogen peroxide causes a hypothyocyanite ion concentration in the range of 500 micromoles per liter to 1000 micromoles per liter.

9. The composition according to claim 1, wherein the pH adjusting
10 agent is selected from the group consisting of organic acids, and their alkali metal and ammonium salts, alkali metal phosphates, alkali metal hydroxides, ammonium hydroxides, amines, and combinations thereof.

10 10. The composition according to claim 9, wherein the organic acids and their alkali metal and ammonium salts are selected from the group consisting of acetic acid, citric acid, malic acid, butyric acid, gluconic acid, adipic acid, glutaric acid and malonic acid and their corresponding sodium, potassium and ammonium salts.

20 11. The composition of claim 9, wherein the amines are selected from the group consisting of triethanolamine and tris (hydroxymethyl)aminomethane.

12. The composition of claim 9, wherein the alkali metal hydroxides are selected from the group consisting of sodium hydroxide, potassium hydroxide,
25 and combinations thereof.

13. The composition of claim 9, wherein the alkali metal phosphates are selected from the group consisting of the sodium, potassium, and ammonium salts of phosphate, pyrophosphate, and polyphosphate compounds.

14. The composition of claim 1, wherein said pH-adjusting agents have a concentration in the range of 100 micromoles per liter and 500 millimoles per liter.

5 15. The composition according to claim 1, wherein the pH adjusting agent provides a pH of between 4.0 and 7.9 in the aqueous solution.

16. The composition according to claim 15, wherein the pH adjusting agent provides a pH of between 5.0 and 6.0 in the aqueous solution.

10

17. The composition of claim 1, further comprising a halide ion selected from the group consisting of chloride, iodide, bromide, thiocyanate, and combinations thereof.

15 18. The composition of claim 17, wherein the thiocyanate ion is one of potassium or sodium thiocyanate, at a concentration of 500 micromoles per liter to about 10 millimoles per liter.

19. The composition according to claim 18, further comprising a
20 peroxidase enzyme at a concentration of about 1000 ABTS units per gram of composition.

20. A process for manufacturing an oral care product, comprising:

- (a) obtaining an alkali metal percarbonate;
- 25 (b) dispersing the percarbonate in a non-hygroscopic material so as to encapsulate the percarbonate;
- (c) obtaining particles of percarbonate encapsulated in the non-hygroscopic material;
- (d) associating the percarbonate particles with a pH adjusting
30 agent; and

(e) formulating the particles into an oral care product.

21. A process according to claim 20, wherein the oral care product is selected from the group consisting of an animal chew, a chewing gum, a lozenge
5 and a toothpaste.

22. A process according to claim 21, wherein the non-hygroscopic material is a fluid or a solid.

10 23. A process according to claim 22, wherein the non- hygroscopic material is a fluid selected from the group consisting of mineral oils, vegetable oils, fatty esters, silicone fluids, fluorinated hydrocarbons, and fluorosilicones.

24. A process according to claim 22, wherein the non-hygroscopic
15 material is a solid selected from waxy solids consisting of mineral oils, vegetable oils, fatty esters, silicone fluids, fluorinated hydrocarbons, fluorosilicones, stearic acid, glyceryl monostearate, paraffin wax, microcrystalline wax, and fatty alcohols.

20 25. A method of activating a peroxidase system in an oral cavity of an animal, comprising:

(a) selecting a non-enzymatic water soluble hydrogen peroxide precursor capable of rapidly releasing an effective amount of hydrogen peroxide for activating the peroxidase system in the oral cavity upon contact with an
25 aqueous solution;

(b) mixing the precursor with a pH-adjusting agent capable of producing a selected pH in the aqueous solution for facilitating the rapid release of the hydrogen peroxide from the hydrogen peroxide precursor, and the activation of the peroxidase enzyme in the oral cavity; and

30 (c) administering to the oral cavity, the precursor and pH

adjusting agent in a suitable formulation.

26. The method according to claim 25, further comprising the step of rapidly releasing at least 100 micromoles per liter of hydrogen peroxide upon
5 contact with an aqueous solution.

27. The method according to claim 25, wherein the pH is in the range of about 4.0 and 7.9 in the aqueous solution.

10 28. The method of claim 25, wherein the composition is associated with an orally-acceptable carrier selected from chewing gum, dental floss, dentrifice, lozenge, animal chew, or animal feed.

INTERNATIONAL SEARCH REPORT

International Application No
PC1/US 96/13240

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K7/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A,2 325 364 (GOUPIL) 22 April 1977 see the whole document ---	1,2, 5-10,13, 14,25, 26,28
X	FR,A,2 325 363 (GOUPIL) 22 April 1977 see the whole document ---	1,2, 5-10,13, 14,25, 26,28
X,P	US,A,5 464 608 (KHARTCHENKO ET AL.) 7 November 1995 see the whole document ---	1-3, 5-10, 14-16, 25-28
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

27 November 1996

Date of mailing of the international search report

06.12.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Fischer, J.P.

INTERNATIONAL SEARCH REPORT

Intern. Application No
PC1/US 96/13240

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,M,7 175 (DR. SCHELLER DURO-DONT) 22 September 1969 see the whole document ---	1-10, 13-16, 25-28
X	EP,A,0 535 816 (COLGATE-PALMOLIVE) 7 April 1993 see the whole document ---	1-10, 13-16, 25-28
X	FR,A,2 327 762 (COLGATE-PALMOLIVE) 13 May 1977 see the whole document ---	1-3, 5-10, 13-16, 25-28
X	US,A,4 980 152 (FRAZIER ET AL.) 25 December 1990 see the whole document ---	1-3, 5-10, 12-16, 25-28
X	WO,A,88 06879 (PEROXYDENT GROUP) 22 September 1988 see the whole document ---	1-10, 13-16, 25-28
X	EP,A,0 332 551 (R.C.D.LABO) 13 September 1989 see the whole document ---	1-10, 13-16, 25-28
X	US,A,5 000 941 (CHERNACK) 19 March 1991 see the whole document ---	1-10,14, 25,26,28
X,P	GB,A,2 290 234 (JANINA INTERNATIONAL) 20 December 1995 see the whole document ---	1-7,9, 10,13, 14,19, 25,26,28
X	US,A,5 403 578 (GORDON) 4 April 1995 see the whole document -----	1-7,9, 25,26,28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/13240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2325364	22-04-77	BE-A- 846602 DE-A- 2643411 GB-A- 1565672	24-03-77 07-04-77 23-04-80
FR-A-2325363	22-04-77	BE-A- 846601 DE-A- 2643410 GB-A- 1563828	24-03-77 07-04-77 02-04-80
US-A-5464608	07-11-95	CA-A- 2081408	27-04-94
FR-M-7175	11-08-69	DE-A- 1617430 BE-A- 701768 CH-A- 492454 FR-A- 1559617 NL-A,B,C 6709563 US-A- 3577521	13-04-72 02-01-68 30-06-70 14-03-69 30-01-68 04-05-71
EP-A-535816	07-04-93	US-A- 5171564 US-A- 5256402 AU-B- 660691 AU-A- 2131292 CA-A- 2077609 CN-A- 1070335 GR-B- 1001363 JP-A- 5194165 NZ-A- 244175 PT-A- 100861 ZA-A- 9206501	15-12-92 26-10-93 06-07-95 18-03-93 14-03-93 31-03-93 29-10-93 03-08-93 26-07-95 30-11-93 28-02-94
FR-A-2327762	13-05-77	GB-A- 1492660 JP-C- 1384154 JP-A- 52054038 JP-B- 61052123	23-11-77 09-06-87 02-05-77 12-11-86
US-A-4980152	25-12-90	NONE	
WO-A-8806879	22-09-88	DE-T- 3786920 EP-A- 0305365	24-03-94 08-03-89
EP-A-332551	13-09-89	FR-A- 2627084	18-08-89

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/13240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-332551		DE-T- 68906061	20-01-94
US-A-5000941	19-03-91	NONE	
GB-A-2290234	20-12-95	NONE	
US-A-5403578	04-04-95	AU-A- 1698695	29-08-95
		WO-A- 9521605	17-08-95